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(57) Abstract

The current invention concerns the use of a receptor from the RZR/ROR receptor family or of a functional fragment thereof in a test of a compound for anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity and the production of a receptor ligand complex comprising said receptor or a functional fragment thereof and a ligand of said receptor. Described is also a method for testing compounds for said activity (screening for ligands) and the active compounds identified therewith.

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SCREENING METHOD USING THE RZR RECEPTOR FAMILY

The current invention concerns the use of a receptor from the RZR/ROR receptor family or of a functional fragment thereof in a test of a compound for anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity and the production of a receptor ligand complex comprising said receptor or a functional fragment thereof and a ligand of said receptor. Described is also a method for testing compounds for said agonists or antagonists (screening for ligands) and the active compounds identified therewith.

Introduction

Small lipophilic substances like retinoic acid (RA), 1,25-dihydroxyvitaminD_3 (VD), thyroid hormone (T3) and steroid hormones regulate a number of developmental and physiological processes in vertebrates and in invertebrates by binding to specific receptors that function directly as transcription factors. These ligand-dependent transcription factors are members of the nuclear receptor superfamily.

The nuclear receptor superfamily also includes structurally related proteins for which no ligand has been identified yet and therefore are referred to as orphan receptors (O'Malley *et al.*, Mol. Endocrinol. (1992), **6**, 1259-1361). Examples of such orphan receptors are peroxisome-proliferator activated receptors (PPARs) and chicken ovalbumin upstream promoter transcription factor (COUP-TFs). Despite large diversity in function, two conserved zinc-finger motifs which are involved in binding to DNA appear in all members of this superfamily.

Recently, a novel orphan receptor family has been identified *via* a reverse transcription-polymerase chain reaction (RT-PCR) strategy (Becker-Andre *et al.*, Biochem. Biophys. Res. Com. (1993), **194**, 1371-1379; Becker-Andre *et al.*, Keystone Symposium, Feb. 7-13, 1994, Taos, New Mexico, p.376). RZR/RORs are able to bind as monomers to their specific response elements, but they seem to interact with certain constellations of binding sites cooperatively as homodimers. RZR/RORs show constitutive transactivation and despite different approaches no ligands have been isolated so far for the RZR/ROR receptor family and therefore, it has been assumed, that RZR/RORs may provide constitutive rather than ligand-inducible transactivation.

Surprisingly, it has now been found, that melatonin is a natural ligand of the RZR/ROR receptor family. It was only known that melatonin is a ligand of a membrane receptor, which has been recently cloned from frog skin (Ebiswawa *et al.*, Proc. Natl. Acad. Sci. USA (1994), **91**, 6133-6137) and from mamalian tissues (Reppert *et al.*, Neuron (1994), **13**, 1177-1185). Melatonin is the major hormon of the pineal gland, but it is also produced in extrapinal tissues. It lightens skin color in amphibians by reversing the darkening effect of MSH (melanotropin). Melatonin is a transmitter of photoperiodic information and is a regulator of seasonal reproductive cycles in photoperiodic animals. It has been shown also that melatonin is involved in thermoregulation and neuroimmunoregulation (Fraschini and Reiter, Eds., Plenum Press N.Y., London 1991).

Melatonin has a short half life in animals and man and it is therefore surprising for melatonin to be a ligand of a nuclear receptor.

As a further surprise, synthetic chemical substances have also been identified as artificial ligands of the RZR/ROR receptor family. Said compounds are known and show anti-autoimmune, anti-arthritic and/or anti-tumor activity (EP-A-494047, EP-A-508955, EP-A-548017, EP-A-548018, CH-511877 and BE-753532). These properties can be demonstrated *in vivo*, for example in the adjuvant arthritis model in rats in accordance with Wiesenberg *et al.*, Clin. Exp. Immunol. (1989), **78**, 245 and the DMBA-tumor model in rats (Schmidt-Ruppin *et al.*, Experentia (1973), **29**, 823-825).

These compounds and the pharmaceutically acceptable salts thereof are known to have valuable pharmacological properties in the treatment of diseases of the rheumatoid type. Those diseases include, especially, rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, and other seronegative spondylarthritises, Colitis ulcerosa and Crohn's disease, and also reactive arthritises, collagen diseases, such as Lupus erythematosus and scleroderma, degenerative rheumatic diseases, extra-articular rheumatic and para-rheumatic diseases, for example gout, osteoarthritis and osteoporosis. Furthermore, compounds of this type have immuno-modulating and anti-tumor activities and, hence, can be administered generally in tumor therapy and in autoimmune based or related diseases such as multiple sclerosis, Hashimoto thyriditis, juvenile diabetes and psoriasis.

Nobody could expect or predict that these compounds function as ligands of the RZR/ROR receptor family, too. The anti-autoimmune, anti-arthritic and/or anti-tumor activity observed, implies that the binding of the ligand to the receptor enhances the affinity of the receptor to

specific DNA regions (so called hormone response elements) in genes, which are involved in the regulation of cell proliferation and/or differentiation. The transcription of these response genes is either up or downregulated after binding of the ligand-receptor complex. Based on this novel observations it is now possible to use this receptor family for the screening of further compounds (ligands) having anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity.

Detailed description of the invention

Thus the present invention preferably relates to the use of a receptor of the RZR/ROR receptor family or a functional fragment thereof in a test for identifying a compound with anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity.

In general, these compounds (ligands) can be tested for agonistic and antagonistic activity in respect to the RZR/ROR receptor family.

The RZR/ROR receptor family embraces nuclear receptors that consist of several domains, each domain having a specific function. Nuclear receptors generally act *via* suppression or activation of transcription after the binding of a ligand to the receptor. Members of the RZR/ROR receptor family show affinity to a certain kind of ligand. Individual members of the RZR/ROR family may, for example, be produced by alternative splicing of a common DNA coding for said receptors.

Functional fragments of these receptors are, e.g., constructs that have the same properties with respect to ligand selectivity, e.g., constructs comprising the ligand binding domain and the DNA binding domain of a receptor of the RZR/ROR receptor family but are devoid of other domains or wherein other protein fragments to address some special properties to the RZR/ROR have been inserted. Also included are fragments that are constructed by combination of the ligand binding domain with other fragments that allow the decision whether a ligand is bound or not as, e.g., another DNA binding domain; or constructs wherein the ligand binding domain is connected *via* a spacer group to a solid carrier for fishing ligands.

Fragments of RZR/RORs including the functional ligand binding domain can also be labeled using one or more groups that can be identified easily, as for example a fluorescent, chemiluminescent or a radioactive group or can be connected to avidin, biotin, a reporter enzyme or any group easily detectable by spectroscopic or immunogenic methods like NMR, IR, UV, NMR, MS and ELISA. Constructs of this type can be used in the screening of compound libraries as described for example in Walter *et al.*, TIBTECH (1993), **11**, 247-254.

Most preferred members of the RZR/ROR receptor family are RZR/RORα, RZR/RORβ or RZR/RORγ (Hirose *et al.*, Biochem, Biophys Res. Comm. (1994), **205**, 1976-1983). RZR/RORs can be used as monomers or dimers including homodimers and heterodimers. Also possible are the splicing variants like RORα1 (Giguere *et al.*, Genes and Development (1994), **8**, 538-553) and the like.

The synthetical ligands known so far, and referred to above, have valuable pharmacological properties in the treatment of diseases of the autoimmune, rheumatoid and/or tumor type.

A further object of the invention is to provide a method for testing compounds for anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity, comprising

a) transfection of a suitable host with an expression cassette coding for a receptor of the RZR/ROR receptor family or a functional fragment thereof;
b) combination of one or more response elements for said receptor and a reporter gene and cotransfection of the host with this construct;
c) addition of the compound to be tested;
d) measurement of the expression of the reporter gene.

Receptors of the RZR/ROR receptor family or functional fragments thereof are as defined above.

Preferably, the host is free from endogenous RZR/ROR or is genetically modified to be free from RZR/ROR before being transfected with the expression cassette coding for a receptor of the RZR/ROR receptor family or a functional fragment thereof. A preferred host is for example a bacterial, fungal as e.g., yeast, insect or mammalian cell. Especially preferred are *Echerichia coli*, *Saccharomyces cerevisiae* and *Drosophila* cells.

Expression cassettes for a receptor of the RZR/ROR receptor family or a functional fragment thereof usually contain a promoter operably linked to a DNA sequence coding for a receptor of the RZR/ROR receptor family or a functional fragment thereof and to a DNA sequence containing transcription termination signals.

Suitable promoter and terminator sequences are preferably chosen to be active in said host and are well known in the art. They can be combined with the structural gene and optionally with a marker group and other favorable elements by standard techniques in genetic engineering.

Nonlimiting examples are: the promoter of the *TRP1* gene, the *ADHI* or *ADHII* gene, acid phosphatase (*PHO5*) gene, *CUP1* gene, isocytochrome c gene, or a promoter of the genes coding for glycolytic enzymes, such as *TDH3*, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), a shortened version of GAPDH (GAPFL), 3-phosphoglycerate kinase (*PGK*), hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, invertase and glucokinase genes, or a promoter of the yeast mating pheromone genes coding for the a- or α-factor.

Transfection protocols for the insertion of an expression cassette, e.g., *via* a plasmid or *via* integration into the genome, are of common knowledge and can easily be adapted to the cassette comprising a receptor of the RZR/ROR family or a functional fragment thereof.

The response elements are known and are usually chosen in accordance with the specificity of the DNA binding domain of the RZR/ROR used in the test. Suitable response elements for the RZR/ROR receptor family include for example the core half site having the DNA sequence $T(^{A}/_{G})GGTCA$.

Preferred are response elements suitable for RZR/RORα, as for example $NANNT(^{A}/_{G})GGTCA$.

The response element(s) is or are usually inserted *via* a short fragment with suitable restriction sites that is, e.g., synthesized on a DNA synthesizer with appropriate ends for the integration in or in the vicinity of the reporter gene using conventional means, as for example cutting the reporter gene with suitable restriction enzymes and inserting the response element. The wording "in or in the vicinity of the reporter gene" stands for a

location capable of influencing the transcription of the reporter gene on binding the receptor. Due to the transcriptional activation properties of RZR/RORs, the response element is preferably inserted at a trans or cis transcriptional activation site of the reporter gene to regulate transcription if a suitable ligand has bound to the ligand binding domain.

The expression of reporter genes can be measured, e.g., on the transcriptional or translational level like the amount of a protein produced, an enzymatic activity or cell growth. Suitable reporter genes are well known in the art. Examples are chloramphenicol acetyltransferase (CAT), β-D-galactosidase (lacZ) or bacterial hybrid luciferase (luxAB).

A different approach in screening compounds for ligand activity is to generate a library consisting of a large amount of different compounds (compound library). The receptors, e.g. RZR/RORs or fragments thereof with an easily detectable group (see above), are incubated with this library and those compounds that are bound to receptors are identified. The construction of compound libraries is broadly described in literature as for example in Jung *et al.*, Angewandte Chemie (1992), **104**, 375-391 and for one bead one sequence libraries in Kerr *et al.*, J. Am. Chem. Soc. (1993), **115**, 2529-2531.

A further embodiment of the invention is a method for the production of a receptor ligand complex comprising incubating a receptor from the RZR/ROR receptor family or a functional fragment thereof with a compound showing anti-autoimmune anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity.

Receptors from the RZR/ROR receptor family or a functional fragment thereof can be obtained as described above, e.g. *via* expression in a suitable host or *via* isolation from the natural surroundings (Becker-André *et al.*, Biochem. Biophys. Res. Com. (1993), **194**, 1371-1379).

The incubation method depends on the method that provides the receptor. If, for example, the receptor is provided *via* expression in a suitable host, as described above, the test compounds can be added directly to the cell suspension or, if the compounds are unable to pass the cell membrane, suitable carriers have to be added or a cellfree system has to be used.

In case of screening compound libraries, the receptor is usually added to a solution or suspension comprising said library. If the receptor is fixed to an insoluble carrier like a

bead, a little rod, foil or cellulose paper, it is also possible to add the compound library to the receptor, e.g. to add the library to a column comprising the fixed receptor.

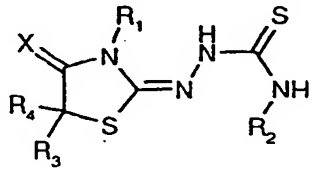
The identification of the compounds that bind to the receptors (ligands) depends on how the compounds and/or the receptors are provided. In case of receptors that are expressed in a cell and that bind to a regulatory sequence of a reporter gene, the compound can be identified *via* the transcription or translation product of the reporter gene; or in case of screening compound libraries, e.g., an one bead one sequence library, the beads having bound receptor can be isolated and the ligand connected to the beads identified.

A further part of the invention concerns the use of a novel ligand that has been identified to bind to a receptor from the RZR/ROR receptor family or a functional fragment thereof as described above, in a method of treatment, especially in a method of treatment of autoimmune diseases, arthritic diseases, tumors, or diseases where usually melatonin or a melatonin agonist or antagonist is applied and more preferred in a method of treatment of autoimmune diseases, arthritic diseases and/or tumors.

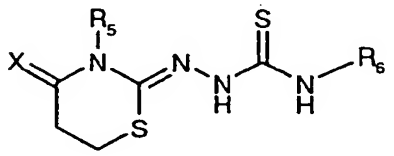
Since there are no ligands with melatonin-antagonistic properties at the RZR/ROR receptor available so far, their pharmacological profile and their potential therapeutic value is currently unkknown.

Suitable ligands of RZR/ROR receptors are, for example, melatonin or melatonin derivatives as described for example in EP-585206.

Further compounds that bind to the RZR/ROR receptor family are for example those of formula (1) to (17)

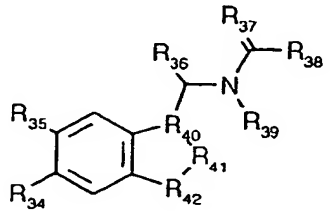


(1)



(2)

wherein R_1 and R_5 are C_3-C_5alkyl, C_3-C_5alk-2-en-1-yl or C_3-C_5alk-2-yn-1-yl; and especially allyl, methallyl and propinyl;
R_2 and R_6 are hydrogen, C_1-C_5alkyl, C_3-C_5alk-2-en-1-yl, C_3-C_5alk-2-yn-1-yl, aryl, aryl lower alkyl, saturated or unsaturated heterocyclyl lower alkyl or lower alkoxy carbonyl lower alkyl;
R_3 and R_4 are each independently of the other hydrogen, lower alkyl or together form lower alkylidene; X is oxo or sulfo; or a compound of formula

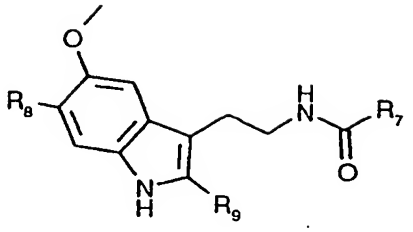


(3)

wherein R_{34} and R_{35} are independent of the other hydrogen, methoxy, or fluoro; R_{36} is hydrogen or methoxycarbonyl, R_{37} is oxo or sulfo; R_{38} is hydrogen, C_1-C_6alkyl, cyclopropyl, cycloputyl, cyclopentyl, cyclohexyl, C_1-C_6alkyl substituted by Br, Cl, F or I, phenyl, C_1-C_3alkyl-benzene, substituted or unsubstituted by halogen, indolyl, morpholino, methylmorpholino, amino, amino substituted with C_1-C_4alkyl, or 1-(2', 3' ,4'-trimethoxy-benzyl)piperazine-methyl, 2-pyrrolidinone; R_{39} is hydrogen, methyl or fluoro; R_{40} is a carbon or nitrogen atom; R_{41} is a carbon or nitrogen atom or a carbonyl group; R_{42} is a carbon, nitrogen or sulfor atom or a vinylene group; the bond between R_{40} and R_{41} my be a single or double bond, with the proviso that it is a single bond if R_{41} is a carbonyl group or R_{40} is a nitrogen atom.

Also embraced are pro-drugs that are metabolized *in vivo* to give a compound as described above.

Methods for the synthesis of these compounds and examples for anti-autoimmune, anti-arthritic and/or anti-tumor activity of these compounds are given, for example in EP-447285, EP-A-494047, EP-506539, EP-A-508955, EP-527687, EP-530087, EP-A-548017, EP-A-548018, EP-562956, EP-578620, EP-A-585206, EP-591057, US-5283343, US-5206377, Depreux *et al.* (J. Med Chem (1994), **37**, 3231-3239), Garrat & Vonhoff (Bioorganic & Medicinal Lett. (1994), **4**, 1559-1565) and Copinga *et al.* (J. Med. Chem. (1993), **36**, 2819-2898). Further examples for suitable compounds are:



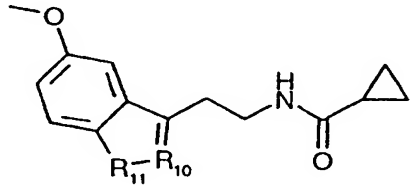
(4)

wherein R_8 = hydrogen; R_9 = bromo; and R_7 = methyl; or

R_8 = hydrogen; R_9 = iodo; and R_7 = methyl; or

R_8 = chloro; R_9 = hydrogen; and R_7 = methyl; or

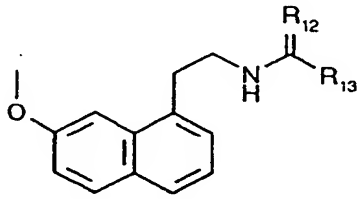
R_8 = hydrogen; R_9 = methyl; and R_7 = chloropropyl; or



(5)

wherein R_{10} = CH; and R_{11} = sulfo or oxo; or

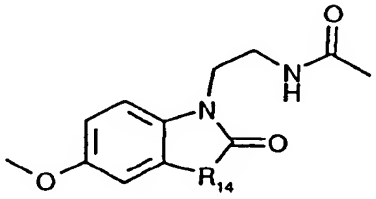
R_{10} = oxo or NH; and R_{11} = NH; or



(6)

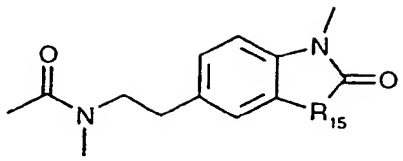
wherein R_{12} = oxo or sulfo; and R_{13} = $NHCH_2CH_2CH_3$; or

R_{12} = oxo; and R_{13} = methyl; or



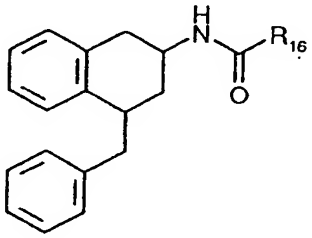
(7)

wherein R_{14} is oxo or sulfo; or



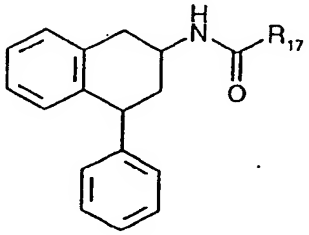
(8)

wherein R_{15} is oxo or sulfo



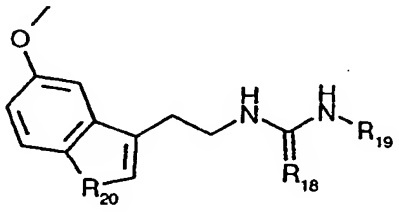
(9)

wherein R_{16} is methly, ethyl or chlormethyl; or



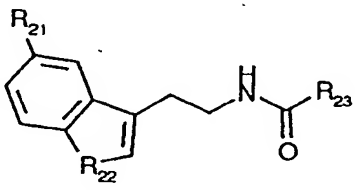
(10)

wherein R_{17} is methly, ethyl or chlormethyl; or



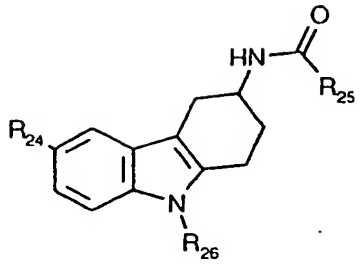
(11)

wherein R_{20} is NH, CH=CH, oxo or sulfo; R_{18} is oxo or sulfo; R_{19} is hydrogen, methyl, ethyl or propyl; or



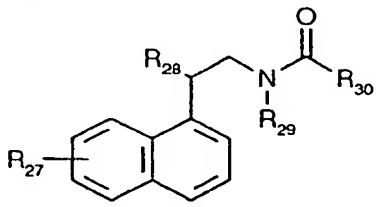
(12)

wherein R_{21} is methoxy or hydrogen; R_{22} is NH, CH=CH, sulfo, or oxo; and R_{23} is methyl, cyclopropyl or cyclobutyl; or



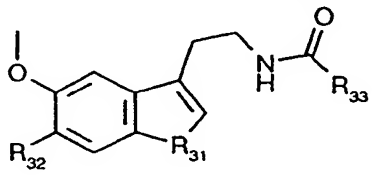
(13)

wherein R_{24} is hydrogen or methoxy; R_{25} is methyl, ethyl, propyl, CF_3, CH_2Br, $CHBrCH_2CH_3$, cyclopropyl, or cyclobutyl; or



(14)

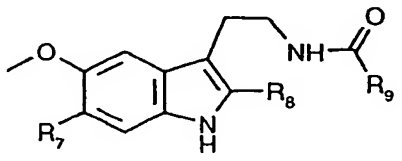
wherein R_{27} is methoxy; R_{28} is hydrogen or $COOCH_3$; R_{29} is hydrogen, methyl or fluoro; and R_{30} is hydrogen, methly, ethyl, butyl, propyl, pentyl, hexyl, isopropyl, $CH{=}CHCH_3$, cyclohexyl, CH_2Br, CH_2I, CF_3, C_3H_6Cl, phenyl, 3,5-di-chlorobenzene, 2-indolyl, toluene, $CH(C_5H_5)_2$, $(CH_2)_2C_6H_5$, $(CH_2)_3C_6H_5$, methyl-morpholino, 1-(2', 3' ,4'-trimethoxy-benzyl)piperazine-methyl, 2-pyrrolidinone, SO_2CH_3; or



(15)

wherein

R_{31} is NH, oxo, or sulfo; R_{32} is hydrogen or fluoro; and R_{33} is propyl, butyl, CH_2I, CF_3 or methyl; or

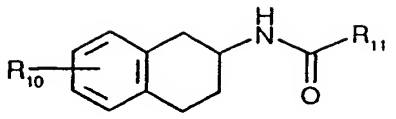


(16)

wherein R_7 = hydrogen or C_1-C_3alkyl;

R_8 = C_1-C_6alkyl, aryl, hydroxy aryl or halogen; and

R_9 = C_1-C_5alkyl or halogen.

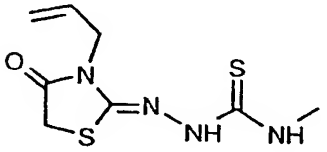


(17)

wherein R_{10} = hydrogen or methoxy; and

R_{11} = C_1-C_3alkyl, aryl, arylalkyl or C_1-C_3alkyl substituted with halogen.

Especially mentioned are compounds of formula (18)-(32)



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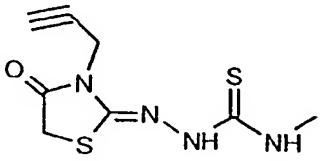

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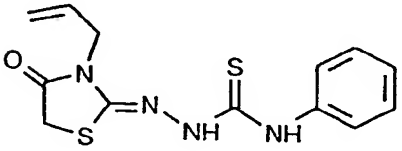

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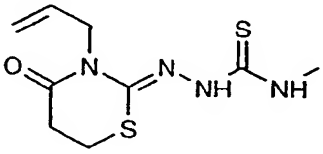

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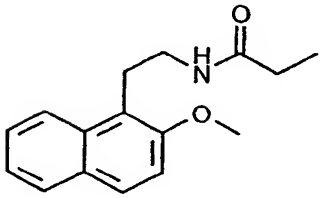

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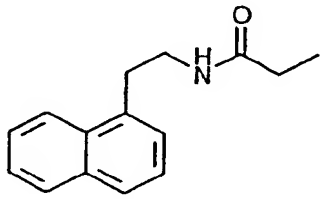

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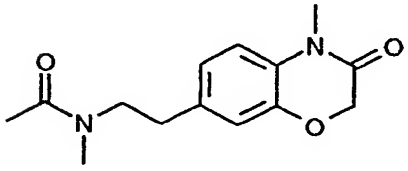

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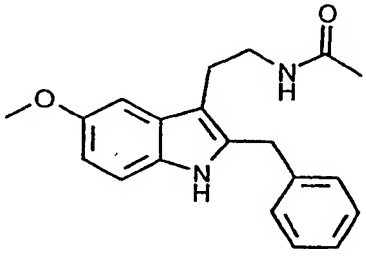

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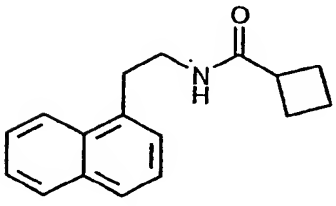

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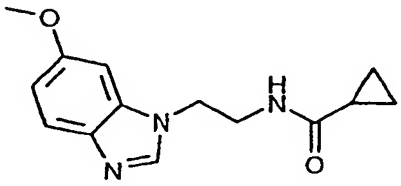

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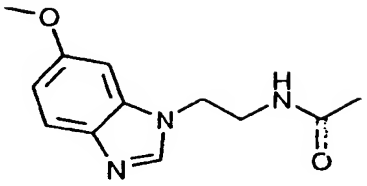

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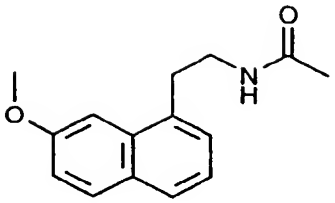

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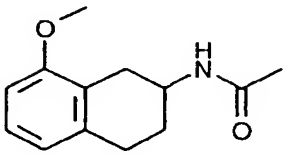

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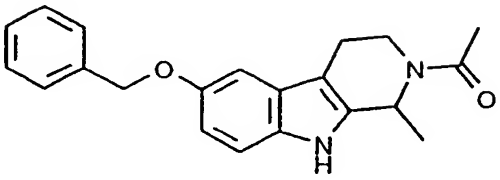

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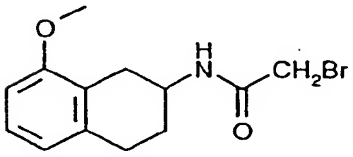

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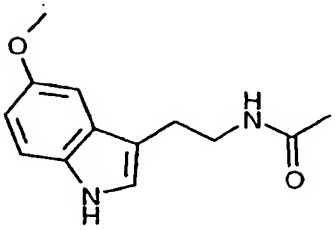
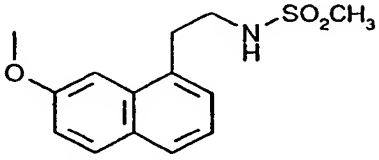


(31)

and



(32)

Also enclosed by the scope of the present invention is the use of a melatonin-antagonist or agonist, preferably, like compounds (1) to (3); more preferably (4) to (17); and most preferably (4) to (10) and (18) to (32); for the modification of the activity of the RZR/ROR receptor family and the use of one or more of these compound as an antagonist or agonist of the RZR/ROR receptor family.

Examples:

Example 1: Construction of the expression system

The test for ligand induced activation is carried out essentially as described in Carlberg *et al.*, Nature (1993), **361**, 657-660.

The response elements for RZR/ROR are cloned as double stranded oligonucleotides with XbaI overhangs into the XbaI site upstream of the tk promoter in the CAT reporter plasmid pBLCAT2 (Luckow *et al.*, Nucleic Acids Res. (1987), **15**, 5490). This insert is synthesized on an automatic DNA synthesizer and the sequences for the response element of the top strand is given in SEQ ID NO:1

GTAGGTCATGACCTAC SEQ ID NO:1

Drosophila SL-3 cells ($2x10^6$ per 60 mm Petri dish; J. Embryol. Expl. Morphol. (1972), **27**, 353-365) are grown overnight in Schneider's medium (Gibco') supplemented with 15 % charcoal-treated fetal calf serum. For transfection, liposomes were formed by incubating 5µg reporter plasmid (with response element insert), 3 µg reference plasmid (pRSVβ-gal) and 1 µg of RZR/RORα expression vector (Carlberg *et al.*, Nature (1993), **361**, 657-660) with 11 µg N-(1-(2,3-di oleolyloxy)propyl)-N,N,N-trimethyl ammonium methyl sulfate (Boehringer-Mannheim) for 15 min. at room temperature in a total volume of 100 µl. After dilution with 0.9 ml Schneider's medium, the liposomes are added to the cells.

Example 2: Test of selectivity

Test compounds (dissolved in ethanol, test-concentration 1 µM, see table 1) that are known to be active or inactive in adjuvant arthritis (see table 1) are added to the cells 8 h after transfection with RZRα. After 40 h the cells are collected for the determination of the CAT activity (CAT assay, a standard technic of genetic engineering, see for example Gorman *et al.* Molec. Cel. Biol. (1982), **2**, 1044-1051). The values given in table 1 represent the mean activation of CAT activities measured in three independent experiments, compared to the addition of pure solvent (ethanol).

To show the specific action of the inventive compounds, the experiments are carried out in parallel with the vitamin D_3 receptor (VDR) and retinoic acid receptors (RAR and RXR) using SEQ ID NO:2 and 3 as response element (Carlberg *et al.*, Nature (1993), **361**, 657-660).

AGAGGTCAAGGAGGTCACT SEQ ID NO:2

AGGGTTCACCGAAAGTTCA SEQ ID NO:3

Table 1:

Compound	Fold stimulation of CAT expression RZRα	VDR	RAR	RXR	activity in adjuvant arthritis	Response element
(3)	3.25	1.2	1.1	1.0	+	SEQ 1
(4)	4.5	1.2	1.2	1.0	+	SEQ 1
(5)	5.2	1.0	1.0	0.9	+	SEQ 1
(6)	5.0	1.2	0.9	1.0	+	SEQ 1
melatonin	4.8	1.1	1.1	1.2	?	SEQ 1
vitamin D	0.9	10.3	1.0	1.0	-	SEQ 2
all trans RA	1.2	1.0	10.4	1.5	-	SEQ 3
9-cis RA	1.1	2.1	1.4	5.3	-	SEQ 3

This clearly demonstrates, that the response of the RZR/RORα is specific for a certain class of compounds.

Example 3: Test of dose response

Various amounts of the test compounds are added to the test system containing compound (3), (4), (6) or melatonin according to table2, RZR/RORα and SEQ ID NO:1 as described in example 2 (see table 2).

Table 2:

Concentration of ligand [nM]	Fold stimmulation using (3)	(4)	(6)	melatonin
1000	4.2	4.1	5.1	4.8
333	4.4	4.0	5.2	5.0
100	4.3	4.2	5.0	5.0
33	4.15	4.1	4.3	5.0
10	2.9	4.2	2.3	3.0
3.3	1.8	4.0	1.4	2.1
1	1.3	2.5	1.1	1.0
0.33	1.1	1.7	1.0	1.0
0.1	0.9	1.3	1.1	1.0
0	1.0	1.0	1.0	1.0

This example demonstrates the high affinity of compounds 3, 4, 6 and melatonin to the RZR/RORα receptor, when applied in the nanomolar range.

Specificity (Table 1) and affinity (Table 2) of the given test compounds were comparable using either the RZR/RORα or RZRβ.

Example 4: Test of recognition sites

The test as described in example 2 is repeated using compound (3) on RZR/ROR and the following response elements:

AGAGGTCAAAAGGTCA	SEQ ID NO:4
TGACCTACTTATAAGTAGGTCA	SEQ ID NO:5
GTAGGTCACTATAAGTAGGTCA	SEQ ID NO:6
TCAGGTCATGACCTGA	SEQ ID NO:7
GTAGGTCATAAGTAGGTCA	SEQ ID NO:8

The increase of CAT activity (see example 1) compared to solvent is given in table3

Table 3:

SEQ ID NO	Fold stimmulation compared to solvent
4	6.8
5	1.75
6	5.8
7	6.2
8	1.8

This example demonstrates that the response is specific for a certain sequence in the response element.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: CIBA-GEIGY AG

(B) STREET: Klybeckstr. 141

(C) CITY: Basel

(E) COUNTRY: Switzerland

(F) POSTAL CODE (ZIP): 4002

(G) TELEPHONE: +41 61 69 11 11

(H) TELEFAX: + 41 61 696 79 76

(I) TELEX: 962 991

(ii) TITLE OF INVENTION: Screening method

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

(A) NAME/KEY: protein_bind

(B) LOCATION: 1..16
(D) OTHER INFORMATION: /bound_moiety= "RZR/ROR"
/standard_name= "P0TT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTAGGTCATG ACCTAC 16

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /bound_moiety= "VDR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AGAGGTCAA GGAGGTCACT 19

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /bound_moiety= "RXR and RAR"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AGGGTTCACC GAAAGTTCA 19

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /standard_name= "CRUBPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

AGAGGTCAAA AGGTCA 16

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..22
(D) OTHER INFORMATION: /standard_name= "IP10"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGACCTACTT ATAAGTAGGT CA 22

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..22
(D) OTHER INFORMATION: /standard_name= "DR8"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GTAGGTCACT ATAAGTAGGT CA 22

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /standard_name= "TREpalP0"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TCAGGTCATG ACCTGA 16

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION: 1..19
(D) OTHER INFORMATION: /standard_name= "DR5GT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTAGGTCATA AGTAGGTCA 19

Claims:

1. Use of a receptor of the RZR/ROR receptor family or a functional fragment thereof in a test for identifying a compound with anti-autoimmune, anti-arthritic, anti-tumor, melatonin like and/or melatonin-antagonistic activity.

2. Use of a receptor according to claim 1, wherein the receptor is RZR/RORα, RZR/RORβ or RZR/RORγ.

3. Use of a receptor according to claim 1, wherein the receptor is RZR/RORα.

4. Use of a receptor according to claim 1, wherein the receptor is a monomer or dimer.

5. Use of a fragment of a receptor according to claim 1 wherein the functional fragment includes the functional ligand binding domain.

6. Use of a fragment of a receptor according to claim 6 that is labeled by one or more groups that can be identified easily.

7. Method for testing compounds for anti-arthritic, anti-autoimmune, anti-tumor, melatonin-like and/or melatonin-antagonistic activity comprising
   a) transfection of a suitable host with an expression cassette coding for a receptor according to claim 1;
   b) combination of one or more response elements for said receptor and a reporter gene and cotransfection of the host with this construct;
   c) addition of the compounds to be tested;
   d) measurement of the expression of the reporter gene.

8. Method according to claim 7, wherein RZR/ROR is RZR/RORα, RZR/RORβ or RZR/RORγ.

9. Method according to claim 7, wherein RZR/ROR is RZR/RORα.

10. Method according to claim 7, wherein the not transfected host is free of endogenous RZR/ROR.

11. Method according to claim 7, wherein the host is a bacterial, fungal, insect or mammalian cell.

12. Method according to claim 7, wherein the host is a yeast or *Drosophila* cell.

13. Method according to claim 7, wherein the response element is specific for RZR/ROR.

14. Method according to claim 7, wherein the response element comprises the DNA sequence $T(^{A}/_{G})GGTCA$.

15. Method according to claim 7, wherein the expression of the reporter gene can be measured on the transcriptional or translational level.

16. Method according to claim 7, wherein the reporter gene is selected from the group consisting of chloramphenicol acetyltransferase (CAT), lacZ and bacterial hybrid luciferase (luxAB).

17. Method for testing compounds for anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity comprising incubating a compound library with the receptor according to claim 1 and selecting the compounds that bind to said receptor.

18. Method for the production of a receptor ligand complex comprising incubating a receptor according to claim 1 with a compound showing anti-autoimmune, anti-arthritic, anti-tumor, melatonin-like and/or melatonin-antagonistic activity.

19. Method according to claim 18, wherein the receptor is RZR/RORα RZR/RORβ or RZR/RORγ.

20. Method according to claim 18, wherein the receptor is RZR/RORα.

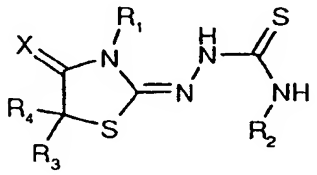
21. Method according to claim 18, wherein the receptor is a monomer or dimer.

22. Method according to claim 18, wherein the receptor is a fragment including the functional ligand binding domain.

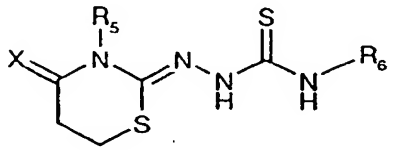
23.Method according to claim 22 wherein the fragment is labeled by group that can be identified easily.

24.Use of a melatonin-antagonist or agonist for the modification of the activity of the RZR/ROR receptor family.

25.Use of a compound according to formula (1), (2) or (3) for the modification of the activity of the RZR/ROR receptor family



(1)



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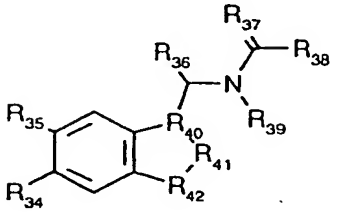

(3)

wherein R_1 and R_5 are C_3-C_5alkyl, C_3-C_5alk-2-en-1-yl or C_3-C_5alk-2-yn-1-yl; and especially allyl, methallyl and propinyl;

R_2 and R_6 are hydrogen, C_1-C_5alkyl, C_3-C_5alk-2-en-1-yl, C_3-C_5alk-2-yn-1-yl, aryl, aryl lower alkyl, saturated or unsaturated heterocyclyl lower alkyl or lower alkoxy carbonyl lower alkyl;

R_3 and R_4 are each independently of the other hydrogen, lower alkyl or together form lower alkylidene;

X is oxo or sulfo;

R_{34} and R_{35} are independent of the other hydrogen, methoxy, or fluoro;

R_{36} is hydrogen or methoxycarbonyl,

R_{37} is oxo or sulfo;

R_{38} is hydrogen, C_1-C_6alkyl, cyclopropyl, cycloputyl, cyclopentyl, cyclohexyl, C_1-C_6alkyl substituted by Br, Cl, F or I, phenyl, C_1-C_3alkyl-benzene, substituted or unsubstituted by halogen, indolyl, morpholino, methylmorpholino, amino, amino substituted with C_1-C_4alkyl; or 1-(2', 3' ,4'-trimethoxybenzyl)piperazine-methyl, 2-pyrrolidinone;
R_{39} is hydrogen, methyl or fluoro;
R_{40} is a carbon or nitrogen atom;
R_{41} is a carbon or nitrogen atom or a carbonyl group;
R_{42} is a carbon, nitrogen or sulfor atom or a vinylene group;
the bond between R_{40} and R_{41} my be a single or double bond, with the proviso that it is a single bond if R_{41} is a carbonyl group or R_{40} is a nitrogen atom.

26. Use of a compound according to claim 24 as an antagonist or agonist of the RZR/ROR receptor family.

27. Use of a ligand identified to bind to a receptor from the RZR/ROR receptor family or a functional fragment thereof in a method of treatment.

28. Use of ligand according to claim 26 in a method of treatment of autoimmune diseases, arthritic diseases and/or tumors.

29. Use according to claim 26, wherein the ligand is melatonin or a melatonin derivative.

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/EP 95/01017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/50 C12Q1/68 C07K14/705 //C07D279:06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 194, no. 3, 16 August 1993 NEW YORK US, pages 1371-1379, M.BECKER-ANDRE ET AL. cited in the application see the whole document	1,7,13
A	--- -/--	2-4,8,9, 17-21, 24,25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
26 June 1995	31. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	De Kok, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 179, no. 3, 30 September 1991 NEW YORK US, pages 1554-1561, G.GRAUPNER ET AL. see the whole document	1,7,13
A	---	11,15,16
A	TIBTECH, vol. 11, June 1993 LONDON GB, pages 247-254, W.H.M.L.LUYTEN ET AL. cited in the application see the whole document	1,5-12, 15-18, 22,23
A	--- BIOTECHNOLOGY, vol. 11, November 1993 NEW YORK US, pages 1256-1261, D.P.MCDONNELL ET AL. see the whole document	1,7,11, 12,14-16
A	--- WO-A-92 16546 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 1 October 1992 see the whole document	1,7,10, 14,17,18
A	--- WO-A-92 16658 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 1 October 1992 see the whole document	7
A	--- EP-A-0 325 849 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 2 August 1989 see the whole document	1
A	--- EP-A-0 085 275 (CIBA GEIGY AG) 10 August 1983	25,26,29
A	--- EP-A-0 585 206 (I F L O S A S DI GIORGIO E ALD) 2 March 1994 cited in the application	25,26
A	--- DE-A-26 32 747 (CIBA GEIGY AG) 24 February 1977	25,26,29
A	--- EP-A-0 508 955 (CIBA GEIGY AG) 14 October 1992 cited in the application	25,26
P,X	--- NUCLEIC ACIDS RESEARCH, vol. 23, no. 3, 11 February 1995 LONDON GB, pages 327-333, I. WIESENBERG ET AL. see the whole document	1-26,29
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/01017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 46, 18 November 1994 BALTIMORE US, pages 28531-28534, M. BECKER-ANDRÉ ET AL. see the whole document ---	1-3
P,A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 205, no. 3, 30 December 1994 NEW YORK US, pages 1976-1983, T. HIROSE ET AL. cited in the application see the whole document -----	1-3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/EP 95/01017

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 27,28
because they relate to subject matter not required to be searched by this Authority, namely:
Reason: Method of treatment of the human or animal body by therapy. see Rule 39.1(iv) PCT.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/EP 95/01017

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		CA-A-	2100584	20-09-92
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		JP-T-	6508509	29-09-94
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		EP-A-	0575528	29-12-93
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		AU-A-	1390692	15-10-92
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